

# UNCLASSIFIED

AD NUMBER
AD457860
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Specific Authority; Other requests shall be referred to Commanding Officer, US Army Biological Laboratories, Attn: TRB/TID, Frederick, MD 21701.
AUTHORITY
BDRL, D/A, Itr, 27 Sept 1971

THIS PAGE IS UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

457860

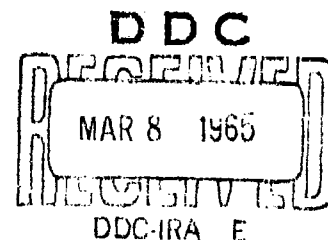
CATALOGED BY (111)

AS AD NO. 457860

# TECHNICAL MANUSCRIPT 196

## BACTERIAL POPULATION NUMBERS IN LARVAL MOSQUITO REARING MEDIA

FEBRUARY 1965



UNITED STATES ARMY  
BIOLOGICAL LABORATORIES  
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES  
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 196

BACTERIAL POPULATION NUMBERS IN LARVAL MOSQUITO REARING MEDIA

H. Ellen Brady  
Robert E. Williams  
Robert E. Freytag  
Dennis Katchur

Entomology Division  
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1C522301A05901

February 1964

This publication or any portion thereof may not be reproduced without specific authorization from the Commanding Officer, U. S. Army Biological Laboratories, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701. However, DDC is authorized to reproduce the publication for U. S. Government purposes.

The information in this publication has not been cleared for release to the public.

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this publication directly from DDC.

Foreign announcement and dissemination of this publication by DDC is limited.

### ABSTRACT

Medium supporting larval Aedes aegypti were sampled for total numbers of bacteria present. Over a period of 12 months 61 lots of media were examined. Bacterial growth rate was no faster in the medium where excessive numbers of larvae were dying than in the "normal" medium. Bacterial cells in both media reached a peak of  $10^8$ /ml of medium.

### BACTERIAL POPULATION NUMBERS IN LARVAL MOSQUITO REARING MEDIA

Many workers have dealt with bacteria in relation to mosquito development. Atkins and Bacot in 1917<sup>1</sup> indicated that bacteria were necessary for stimulating hatch. However, Barber in 1928<sup>2</sup> and Hinman in 1930<sup>3</sup> were able to rear sterile cultures of mosquito larvae. Rozeboom in 1934<sup>4</sup> was able to reconcile these two opposing views by explaining that it appeared that the condition of the eggs determined their hatchability.

Hinman,<sup>3</sup> who studied the food of mosquito larvae, believed that growth of Bacterium coli, Staphylococcus albus, or Pseudomonas fluorescens produced toxic products that caused the death of second instar larvae of Culex spp. He justified this belief by showing that these bacteria, when inoculated into autoclaved pond water (a medium that did not support rapid bacterial multiplication), supported larval development. Rozeboom in 1935<sup>5</sup> found that the best larval development occurred in suspensions of "environmental bacteria," i.e., Escherichia coli, Bacillus subtilis, Bacillus mycoides, Aerobacter lactis-aerogenes, and Pseudomonas fluorescens. He also found that Sarcina lutea was of little food value, and larvae died rapidly in media contaminated with Pseudomonas pyocyanus because, he postulated, of toxic products formed.

Reduced yields and occasional complete loss of mosquito larvae during the rearing process hampered our research to the extent that a positive research program with the ultimate goal of defining and controlling this problem was initiated. Since the excessive larval mortality might have been due to any number of causes, we chose as our first step to investigate bacterial contamination. We approached the problem by determining the effect of total numbers and rate of growth of the bacteria naturally occurring within the media, without regard to kinds of bacteria present.

The larvae used in this study were from a strain of Aedes aegypti obtained from Rockefeller Institute. They were fed daily on a diet of finely ground, autoclaved dog chow and received a suspension of 22% live yeast approximately 72 hours after they were added to the rearing tray.

Samples of the media were obtained from the rearing trays immediately before the larvae and initial food were added, and one hour, 24, 25, 48, 49, 72, 73, 96, and 97 hours thereafter. These samples were treated with 0.01% actidione to inhibit yeasts and mold that might have been present. Serial dilutions were then made and routine plate counts were obtained.

A total of 61 replicates from 23 lots of 6 rearing trays per lot were analyzed over a period of twelve months of sampling. In that time only four of the replicates showed excessive larval mortality.

In those trays that showed "normal" (Table 1) development the mean log number of cells per ml started at  $0.18 \pm 0.83$ . One hour after the addition of the food and the larvae, hereafter known as seeding, the count rose to  $0.78 \pm 1.55$  mean log number of cells/ml rearing media. Twenty-four hours after seeding the count had risen to  $6.52 \pm 0.63$ . From this point on, feeding did not make a significant difference in the cell count. The count rose steadily to  $8.00 \pm 0.32$  at 49 hours, plateaued, and showed a slight decline in the 97-hour sample. Figure 1 shows the growth curve for these "normal" trays. This curve resembled that of typical bacterial population as shown in Fig. 2.

TABLE 1. LOG CELL COUNT

	Hour									
	0	1	24	25	48	49	72	73	96	97
N	57	57	57	57	57	54	54	55	50	53
s.d.	0.83	1.55	0.63	0.61	0.35	0.32	0.31	0.37	0.31	0.36
s.e.	0.08	0.10	0.07	0.06	0.02	0.04	0.02	0.03	0.03	0.03
Median	0.33	2.43	6.52	6.82	7.80	8.02	7.96	7.98	7.85	7.68
Mean	0.18	0.78	6.52	6.95	7.70	8.00	7.99	7.91	7.86	7.64
Mode	0.30	0.30	6.50	7.10	7.37	7.99	7.96	7.97	8.06	7.69

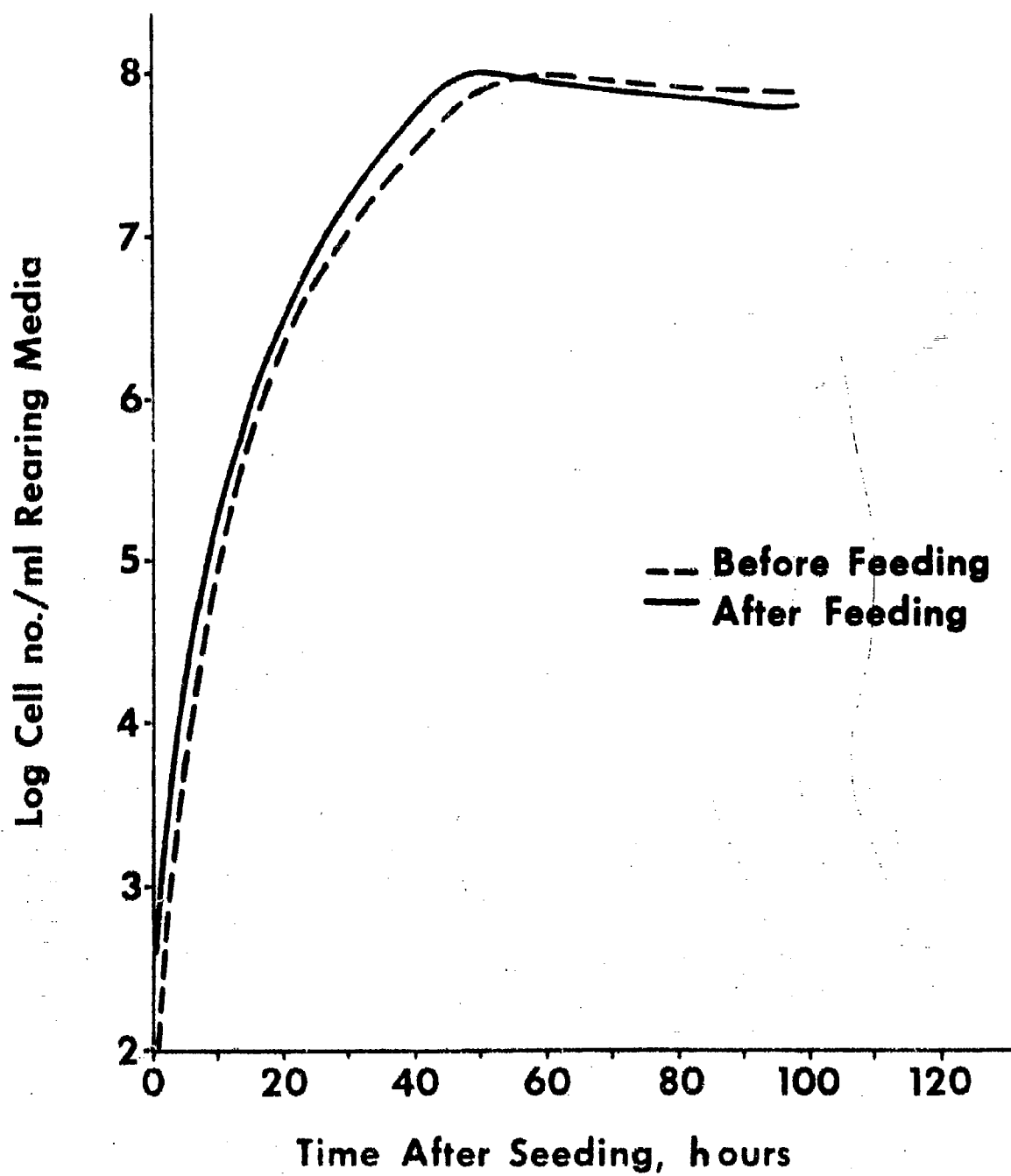


Figure 1. Mean Log Cell Number of Bacteria in Rearing Medium before and after Feeding.



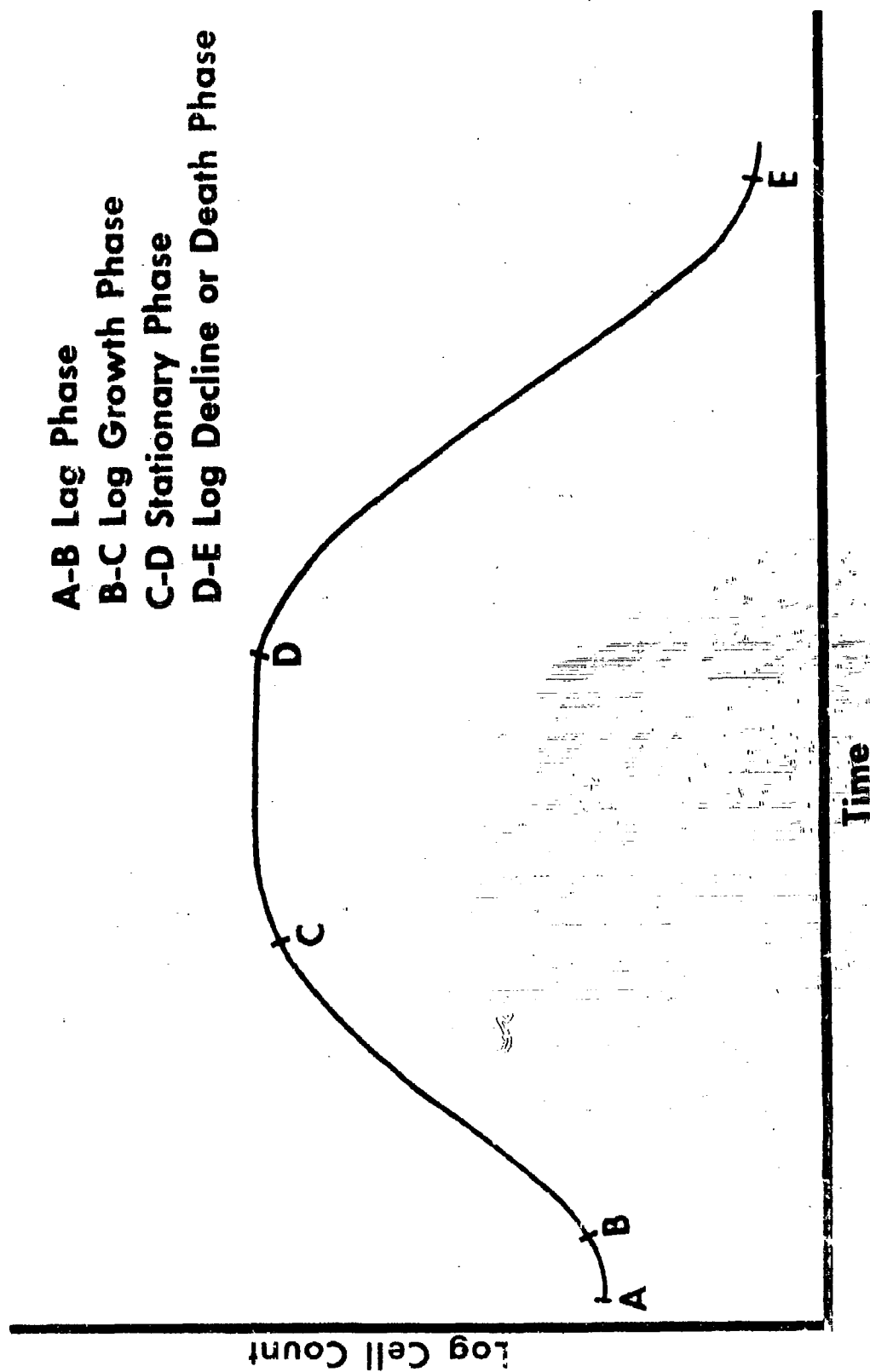


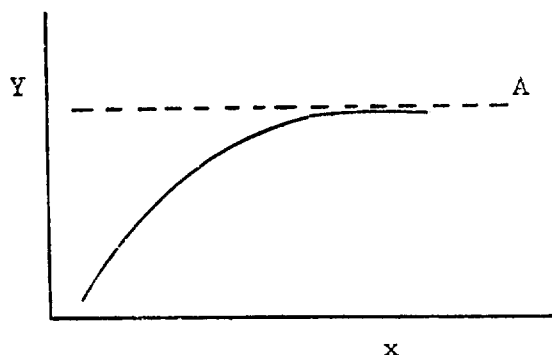
Figure 2. A Typical Growth Curve of a Bacterial Culture.

The data in Fig. 1 were fitted to the Mitscherlich equation. This equation was found to adequately represent the increase in number of organisms during the developmental days' samples.

#### Mitscherlich's Equation

$$Y = A[1 - 10^{-c(x+b)}]$$

Y is the log number of organisms per milliliter of rearing medium  
 A is the logarithm of the theoretical limiting concentration  
 c is related to the rate at which the number of organisms increases  
 x is the developmental day  
 b is related to the intercept



Bacterial growth rates were relatively constant from sample to sample throughout the year. With the exception of the first developmental day, the differences between samples taken before and after the daily addition of larvae food to the trays were not statistically significant, indicating that the larvae food was not a source of increased numbers of bacteria. The only possible clue to an association of contamination with mortality is that the four trays in which high larval mortalities developed were those observed at different seasons. There were slightly higher total numbers of mortalities in winter and slightly lower total numbers in the summer. Other experimental results indicated that neither varying the time of addition of yeast to the rearing media nor aseptically handling the food up to the time it was added to the rearing media affected the bacterial population in the trays. Adding a double amount of yeast to the larval media on the day the larvae were placed in the trays rather than the usual four days later gave rise to a shorter logarithmic growth phase by about one-half. It did not however, alter the total number of bacteria present. It appears that some factor other than total numbers of bacteria was the primary cause of excessive larval mortality.

An examination of the limiting concentration showed that during this period the log concentration of cells was higher in the period December through March than in the period July through October. The rate of increase of cells was not seasonally variable during the total period. Fig. 3 shows the log concentration of cells. The broken line represents a three-date moving average that shows the seasonal variation. Fig. 4 represents the rate of increase. The broken line again is a three-date moving average and indicates that there is no seasonal variation in this function of growth.

Only four replicates over the twelve-month period showed high larval mortality. This was estimated to range from 60 to 80%. The data from these trays indicated that total numbers of bacteria were not the single causative factor of excessive larval mortality; all four fell within the mean  $\pm 2$  s.d. of the normal trays.

Indications that the mortality was seasonal were not substantiated. Fig. 3 shows that the mortalities were not seasonal but occurred during the high-to-low swing of the curve.

o Observed Values  
 x Mean Value for Indicated Date  
 ■ Trays Exhibiting High Mortality  
 --- Three-Date Moving Average

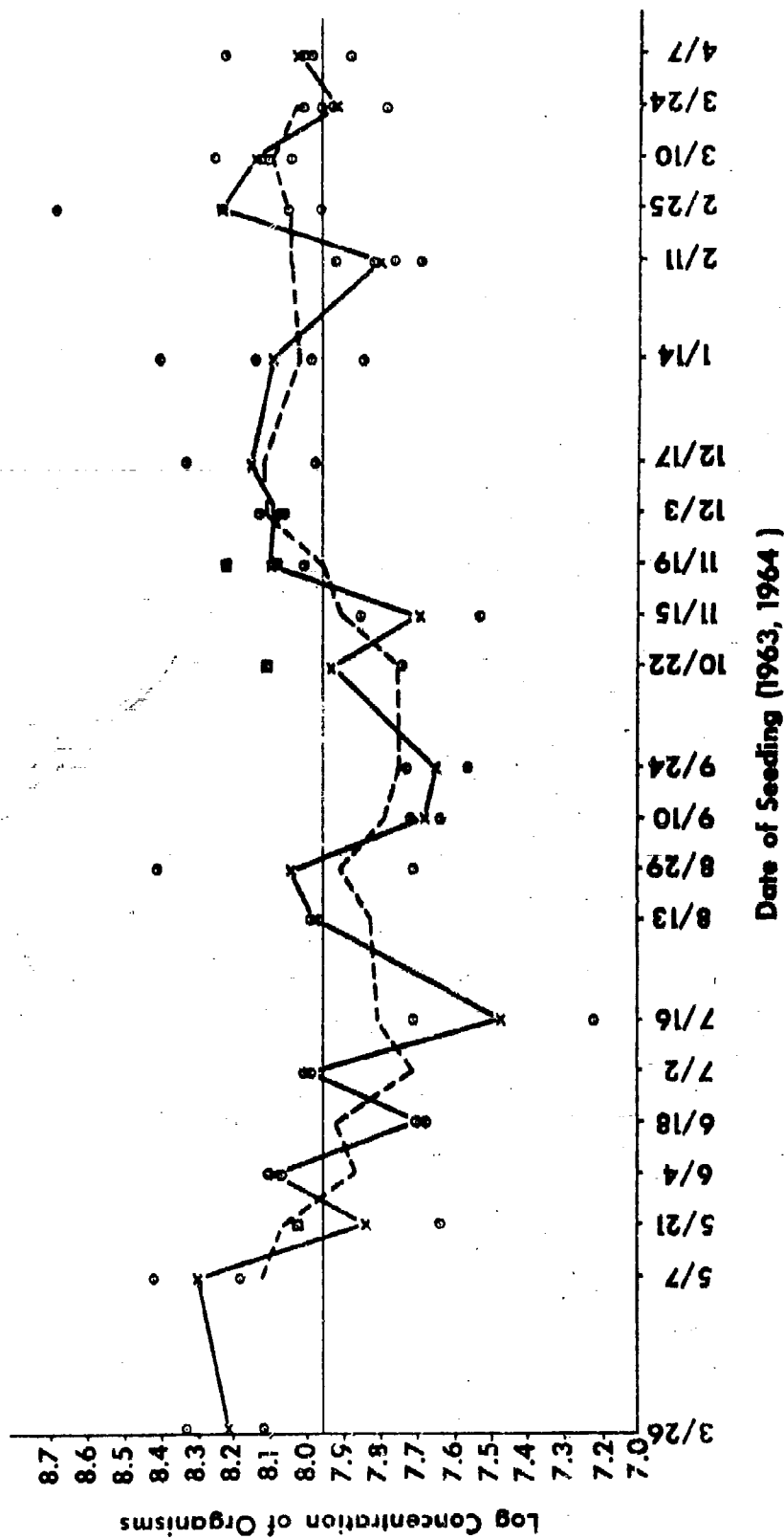


Figure 3. Theoretical Limit of Log Concentration of Organisms  
 ( $\hat{A}$ ) from the Equation  $Y = A[1 - 10^{-c(x+b)}]$ .

○ Observed Values  
 × Mean Value for Indicated Date  
 □ Trays Exhibiting High Mortality  
 --- Three-Date Moving Average

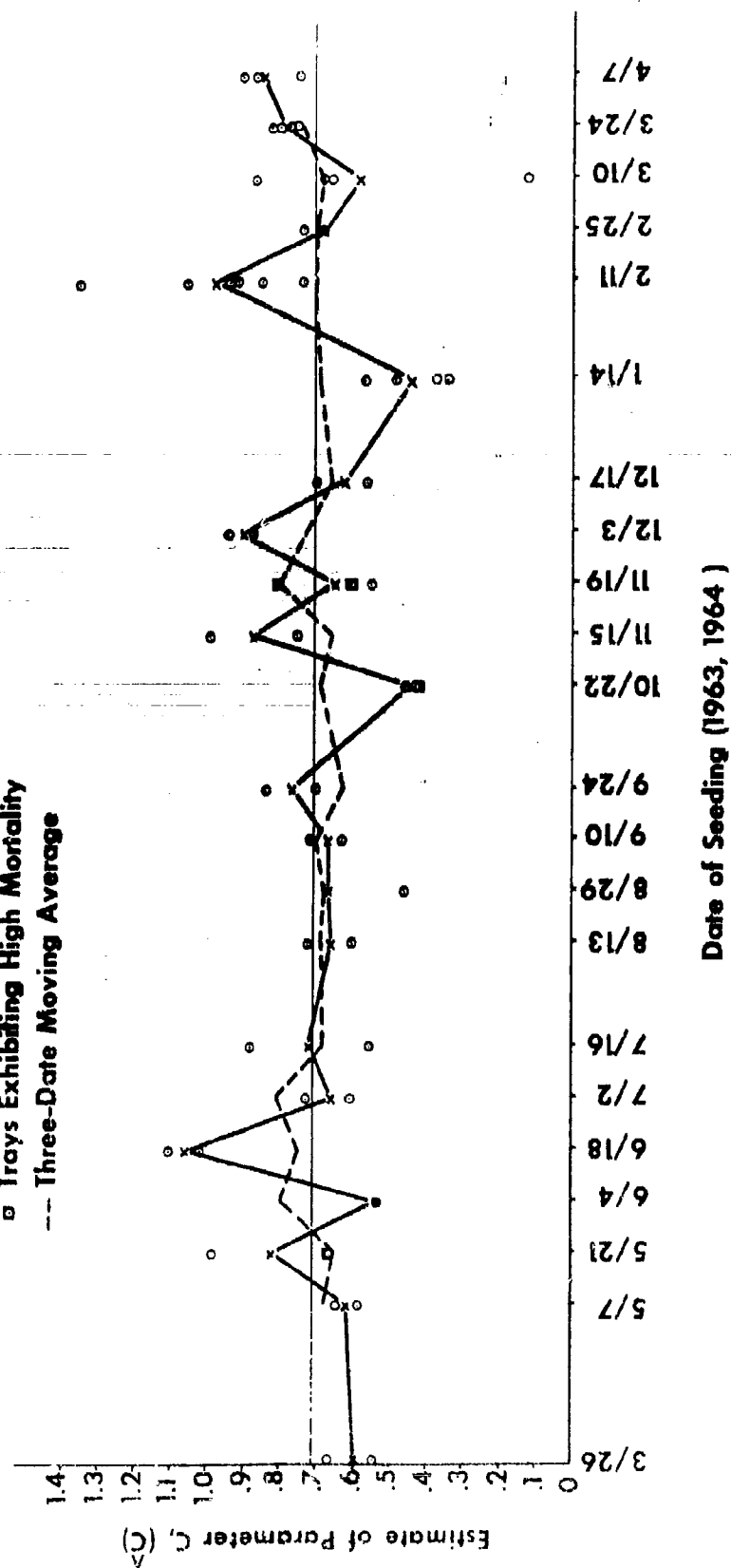


Figure 4. Estimates of Parameter C, (C) Related to Rate of Increase in Number of Organisms.

LITERATURE CITED

1. Atkin, E.E., and A. Bacot. 1917. The relation between the hatching of the eggs and the development of the larvae of Stegomyia fasciata (Aedes calcopus) and the presence of bacteria and yeasts. *Parasitology* 9: 482-536.
2. Barber, M.A. 1928. The food of Culicine larvae. *Public Health Rep.* 43:11-17.
3. Hinman, E.H. 1930. A study of the food of mosquito larvae (Culicidae). *Amer. J. Hyg.* 12:238-270.
4. Rozeboom, L.E. 1934. The effect of bacteria on the hatching of mosquito eggs. *Amer. J. Hyg.* 20:496-501.
5. Rozeboom, L.E. 1935. The relation of bacteria and bacterial filtrates to the development of mosquito larvae. *Amer. J. Hyg.* 21:167-179.